

The University of Chicago

THE NATURE OF THE PULMONARY
ALVEOLAR LINING

A DISSERTATION SUBMITTED TO THE
FACULTY OF THE DIVISION OF THE BIO-
LOGICAL SCIENCES IN CANDIDACY FOR
THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ANATOMY

1933

By

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THE NATURE OF THE PULMONARY ALVEOLAR LINING ¹

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THREE PLATES (FIVE FIGURES)

INTRODUCTION

One of the most recent textbooks of histology (Maximow-Bloom, '34) seriously questions the existence of an epithelial lining for the respiratory portions of the mammalian lung. However, articles constantly appearing as well as a considerable body of histologic tradition uphold the presence of this structure. This tradition goes back nearly three-quarters of a century and has become firmly entrenched in histologic writings.

The older literature has recently been well reviewed by Miller ('32), and the newer by Cappell ('29) and Seemann ('31). Munk (1862) quotes W. Addison (1842) and Schultz (1850) as having previously described the presence of a lining epithelium for the respiratory portions of the mammalian lung, and Rainey (1855) and Zenker (1861) as denying it. He himself attempted the technique then recently advocated by von Recklinghausen (1862) for studying cellular tissues by means of impregnations with silver nitrate. He failed in this and denied the presence of an alveolar epithelium. Eberth (1862) and Hertz (1863) by means of vascular injections with colored masses, however, thought they demonstrated its presence. Hertz attempted, as had Munk, the use of the von Recklinghausen silver technique, but he too reported a failure to get convincing results. The same year

¹ This paper was presented in partial fulfillment of the requirements for the Ph.D degree.

Chrzonaszczewsky (1863) varied the technique a bit and immersed inflated lungs in weak AgNO_3 solutions. His results showed a continuous lining for the respiratory surface consisting of polygonal squamous cells completely covering the alveolar walls. He attributed the failure of earlier workers to get clear pictures by the silver method to their technique. Elenz (1864) and Eberth (1864) by intratracheal injections of silver nitrate solutions into the lungs of experimental animals then produced their classical pictures of what Kölliker later called the respiratory epithelium. Their work also showed a continuous alveolar epithelium, as had Chrzonaszczewsky's. But contrary to the views of the latter, they described two types of component cells; one of small polygonal, nucleated elements forming groups in the interstices of the capillary mesh; the other of large thin protoplasmic squames covering the capillaries and connecting into a continuous whole the scattered groups of nucleated cells. They considered that Chrzonaszczewsky had mistaken pleural mesothelium for lining alveolar epithelium. Villemin (1866) studied preparations of vascular injections of lungs and stoutly denied the presence of an epithelium in the respiratory portions of the lung. He did not use the silver method and was apparently unaware of the work of Elenz and Eberth.

Later, Kölliker (1881) added the weight of his name to the views of Elenz and Eberth and described for the human lung, on the basis of intratracheal silver nitrate injections, likewise a continuous epithelium consisting of small nucleated cells and large non-nucleated plates. He gave to this tissue the name respiratory epithelium. His views appeared later in his *Handbuch* (1899) and have come to be regarded as classic for the finer structure of the respiratory portions of the lung. Much later Ogawa ('20) on an extensive series of laboratory animals duplicated the observations and reasserted the views of Elenz, Eberth and Kölliker.

But while this concept has come to be classic and has appeared in most textbooks, it has been questioned from time to time. Miller ('32) champions the older views of Chrzonasz-

czewsky and, denying the existence of non-nucleated epithelial plates, attempts to demonstrate his opinions by picturing the sloughing of such epithelium in pneumonias and other pathologic processes in the lung. Lange ('09) attempted by staining washings recovered from the lungs of experimental animals to demonstrate non-nucleated plates. His washings contained every cell type excepting the one desired. Maximow-Bloom ('34), Bratianu and Guerriero ('31) and Seemann ('31) doubt that the lines seen on alveolar walls in silver impregnations of lungs should be interpreted as cell limits bounding non-nucleated plates. Policard ('26, '29) is vehement in denial of the presence of anything which might be termed alveolar epithelium. Finally, there has never appeared a description of non-nucleated plates in sputum from patients with lung disease.

The identity of the small nucleated epithelial elements of the Kölliker theory has been the subject of a tremendous amount of literature. From the time of Slavjansky (1869), and before, the presence in relation to the pulmonary alveoli of cells capable of the ingestion and removal of foreign substances has been of great interest. With the introduction of the use of vital dyes in studies on phagocytosis by Goldmann ('12), and others, fresh impetus was given to such studies and their numbers increased. The result of this work has been that most workers now recognize in the walls of the pulmonary alveoli the presence of cells which respond with avid phagocytosis to the introduction of substances into the trachea. These cells have been thought to be derived by different workers from four main sources: the lining alveolar epithelium; locally resident histiocytes; cells emigrated from the pulmonary blood vessels; the vascular endothelium. Seemann ('31) has a reasonably complete account of this discussion and of the conflicting views of the several workers. Suffice it to say that there is a growing tendency for later workers to identify these cells with the small nucleated epithelial cells revealed by the silver methods. Lang ('26) was the first to suggest their possible histiocytic nature. He also

introduced the term 'septum cells,' in describing them. Others have since questioned their epithelial characters and likewise suggested that they may be merely connective tissue histiocytes (Fried, '27, '28, '31; Gardner and Smith, '27).

Since the traditional views of lung structure have been thus questioned, it was here determined to attempt by modern histologic methods further test of their validity. By the careful preparation of normal adult lungs and others of an experimental nature it was hoped to show that a respiratory epithelium either in the sense of Elenz-Kölliker or of Chrzonszczewsky-Miller might be demonstrated.

Now let us define the terms already used and those subsequently to appear in this paper. By "respiratory portions of the lung" are here meant those parts below the respiratory bronchioles designated in the BNA ductuli alveolares, sacculi alveolares, and alveoli. There should be included in this category also those alveoli which open out of respiratory bronchioles.

METHODS AND OBSERVATIONS

Adult lungs

Some early work was done in repetition of some experiments of Foot ('23) which it is not the present intention to describe at great length. This consisted of the injection into a series of normal adult rabbits of colloidal dye suspensions consisting of India ink, trypan blue, and lithium carmin, via the trachea or via the vascular system, as has been done several times since by Fried ('27), Cappell ('29), and others. These lungs were prepared by opening the chests, snipping off bits of lung with scissors and fixing by immersion. Sections of this material were cut in the usual way to 4μ or 5μ and studied. They revealed, in the case of the intratracheal injections, the presence of large numbers of cells in the alveoli, some free in the lumens, others attached to the walls, the greater number of which contained dye particles stored after the manner of histiocytes. The intravascular injections showed fewer cells containing dye, most of which remained

lodged in the alveolar walls, but again storing dye after the same fashion. However, this technique was deemed not suitable for solving the problem of the nature of the cells of the alveolar wall because quite different conclusions have been and might again be drawn from the above set of observations. Also the method of fixation of these lungs was not considered good, and a little reflection will show the validity of the criticism made of it below. Subsequently, therefore, lungs were prepared by the methods later to be described.

Because of its vital importance I feel that the matter of technique in the preparation of the lung should be mentioned, although the point has already been well made in Maximow-Bloom ('34). Thus, adequate fixation of lung tissue is not to be accomplished by the ordinary means. Since the lung normally functions in a space whose pressure is well below that of the atmosphere and since it consists of myriads of delicately walled tubes and chambers whose volumes change with each respiration, it must be obvious that if the chest be simply opened and a bit of tissue cut away and immersed in fixative great distortion must result. Nor is it sufficient to remove from the body both lungs with the trachea and subsequently distend them via the trachea, for such procedure must as obviously stretch and tear them. Thus, in addition to the usual precaution for fixation, viz., assurance of the penetration of the fixative, here must be added those of prevention of collapse and of support for the flimsy structure. These things may be accomplished in several ways. The trachea may be cannulated and known gentle air pressures applied, after which the trachea may be securely tied and the lungs removed in toto and immersed. Or lungs inflated as described may subsequently be perfused before immersion. Finally, with the lungs in situ fixative may be poured under the gentlest possible pressure into the cannulated trachea, which is later tied. In all of these procedures care must be taken to stop the inflation short of forcing the lungs to fill the chest completely, that over-distention may be avoided.

The latter may also be avoided by securely ligating the trachea before opening the chest—but this is likely to give insufficient inflation. Of the above methods those of perfusion and intra-tracheal fixation seem to be best. Maximow-Bloom ('34) also stresses the value, in lung studies, of the use of thick sections. I must re-emphasize this, for one who has not studied preparations of lungs cut to the order of 100μ cannot realize their value, for they give a third dimensional picture of what in ordinary thin histologic section is merely a complex lacework of wide open spaces and thin tissue partitions. This depth picture is necessary to an understanding of the true structure of this extremely complex organ.

Cells

For the study of the cells of the pulmonary alveolar wall the following methods were used. The lungs of adult rabbits were prepared in an inflated condition, either by the introduction of air or of fluid via trachea. The animals were killed in one of two ways. About 1 cc. of a 50 per cent solution of sodium nitrite was injected into an ear vein, in which case death usually occurred in about 5 minutes. Or the animals were put into a bell jar into which illuminating gas was then passed. In this case death usually occurred about as rapidly as in the first. These methods also have the advantage that the blood is kept from clotting for some time, and in the first some vasodilatation is secured as well. The method of asphyxiation by illuminating gas was not long used, however, for it was soon found that severe pulmonary hemorrhages sometimes occurred rendering the lungs unfit for use as normal specimens.

Where air inflation of the lungs was desired the organs were left in situ, the trachea cannulated, and pressure bottles applied. These were set up as follows: Two wide-mouth 12-ounce bottles were partly filled with water and stoppered with tightly fitting corks, each bored for two small glass tubes. One of these tubes was passed just inside the cork of each bottle. The other was passed in far enough to almost

reach the bottom of each. The long tubes were connected with rubber hose, and upon elevating one of the bottles a siphon was started which forced air out of the lower bottle as water poured in. A tube connecting the outlet of this bottle with a tracheal cannula thus delivered a stream of air of measurable force. A height of 4 to 5 inches was found ample to gently distend the lungs of a rabbit.

Upon inflation the trachea was tied securely before releasing the pressure apparatus. The lungs were removed in toto from the chest, after cutting away the sternum and anterior ends of the ribs, by grasping the trachea and lifting it while severing the restraining vessels and soft tissues with scissors. They were then immersed whole in fixative, and kept submerged by weighting with a small piece of absorbent cotton. After sufficient fixation a sharp blade was employed to cut slices which were then dehydrated in graded alcohols and embedded in celloidin or paraffin. Such blocks were sectioned at 40μ to 100μ , depending upon the lung, for routine study. In some cases thin sections (4μ) were also made.

Fixation was secured by immersion of air-inflated lungs in formol-Zenker, by intratracheal introduction of formol-Zenker and subsequent immersion in it, and by vascular perfusion followed by immersion in it. Two blocks, from the lungs of a cat and a mink, respectively, fixed intratracheally by Bensley's fluid and embedded in celloidin, were given to me by Dr. R. R. Bensley. Mallory's connective tissue stain was used routinely, but sections were also stained in some cases with M. Heidenhain's azan modification of it. Since, however, the pictures of structure presented by these methods of fixation and staining do not vary except in regard to alveolar pores, the methods will be described as one, and the pores considered later.

Thick sections of such lungs are very illuminating. They reveal a vast honeycomb structure, penetrated by the thick-walled bronchi, bronchioles and blood vessels. Also, unlike thin sections, there are to be seen here fairly flat surface views of alveolar walls, lying parallel to the plane of the

section. These septa, viewed from their surfaces, are our principle concern here. In lungs from which the blood has not been washed such septa appear very cellular. Owing to the closely packed condition of the capillaries great difficulty is encountered in attempting to discover the identities of the cells seen. However, in lungs from which the blood has been pretty well washed, a very different picture is obtained. Here the cells are not very numerous and occasionally whole septa are to be seen in which all of the nuclei are of one character. These nuclei belong to the capillary endothelium, as will be shown later. They are not quite identical in appearance with those of the larger vessels, but are less elongated, much more elliptical, and stain more palely. Usually, however, there is present another cell type. This has a more rounded nucleus and a deeply staining, somewhat granular appearing cytoplasm. Cells of this type are not very numerous in normal lungs and rarely may more than two of them be seen in a single septum. These elements appear identical with those sometimes seen lying free in the alveolar lumens of normal lungs. There seems little doubt that they are also identical with the septum cells of Lang ('26), and also with the nucleated epithelial elements of the older writers. In the alveolar walls themselves these are the only two cell types which can be distinguished.

Capillaries

For the study of the capillary network of the alveolar wall two methods were used. It was discovered that if the vessels were first perfused with a solution of silver citrate and subsequently Mallory's stain applied to the sectioned material the capillary network was outlined in a surprisingly complete manner. As this method had the additional advantage of leaving the vessels transparent it was of decided value in the study of the extent of the capillary bed of the alveolar septa. However, other preparations were made by perfusing the lung vessels with a solution of carmin gelatin containing potassium iodide after the formula of Tandler, to keep the

gelatin from congealing. For these preparations young adult rabbits were chosen. They were killed, their chests opened, their tracheas cannulated and gentle air pressures applied by the apparatus described above. The pulmonary arteries were cannulated and small volumes of isotonic sodium chloride solution containing about 0.2 per cent of sodium nitrite perfused. This was followed by the carmin gelatin solution. For the perfusion of the washing fluid the pressure bottle was kept about 8 inches above the level of the table, while to get penetration of the carmin gelatin into the finer vessels the height had to be increased to about 2 feet. Upon completion of the perfusions the tracheas were tied tightly and the lungs removed in toto and immersed in 10 per cent formalin. After 24 hours they were cut into slices, dehydrated in graded alcohols and embedded in celloidin. The blocks were sectioned at 75μ to 90μ for study. It was found that a weak solution of Delafield's hematoxylin (2 mm.: 30 cc. water) would stain the cell nuclei of such sections without diminishing the contrast value of the carmin gelatin.

The latter preparations, counterstained as indicated, showed a vast reticulated network of thick red lines, running everywhere, branching and anastomosing freely. These are the capillary vessels of the alveolar walls, forming a latticework as seen in surface view, whose area upon measurement is three to five times as great as that of the interstices. Cell nuclei appear in blue against this red background and their scarcity is again worthy of note. Frequently all but one or two of them lie closely applied to the surfaces of the gelatin masses of the capillaries of a septum so as to be easily identified as endothelial cell nuclei. Other nuclei, whose identities are not to be recognized in this technique, are so few in number in most cases that they appear quite incidental.

The sections of lungs perfused with silver citrate and counterstained with Mallory's, while appearing quite different, reveal the same findings on study. The capillary network, while transparent, is quite distinct, and appears of the same extent. Endothelial nuclei are easily distinguished within the

confines of the outlined vessels and, as noted above, comprise the majority of the nuclei seen in most alveolar septa.

Fibers

For the study of the fibers of the alveolar walls thick sections of adult lungs were stained with the Foot modification of the Maresch-Bielschowsky technique, with Mallory's connective tissue stain, and with the orcein method of Unna. The reticular network, as revealed in preparations by the Foot method, consists of a richly branching net of black stained fibrils. They run through the alveolar septa in all directions, anastomosing freely, and contribute a considerable feltwork to the support of the vessels. The nets of the alveolar walls continue directly into those which support the distributing blood vessels and larger air passages. Only in over-distended lungs are these fibers anything but wavy in appearance. In some cases in which the impregnations had for some reason failed of completeness, the usual black lines are much fewer in number and often appear broken. After running for short distances through alveolar walls they end abruptly, their later courses sometimes further indicated in black—often dotted—after unimpregnated gaps. Such sections resemble others, to be described later, prepared by the classical technique of intratracheal silver nitrate injection.

At the mouths of alveoli where the latter open out of alveolar ducts and alveolar sacs, and also sparsely elsewhere in the septa, are seen some larger fibers also staining black in the Foot technique. These not infrequently branch, and in sections stained by Mallory's method they color deeply with the aniline blue. The 4-mm. lens does not resolve in them any component fibrils, and while probably collagenous their identification is not quite certain. But whatever their nature they seem to serve the heavier scaffolding functions of the septa.

In sections stained with orcein after the method of Unna the elastic network is visible. It also consists of a considerable feltwork, though apparently of somewhat less extent than

the reticular. The fibers are quite straight, branch repeatedly at acute angles, and run sometimes for long distances through the substance of adjacent septa. They vary greatly in size, from bare visibility with the 4-mm. lens to easy visibility with the 16-mm. Like the large, possibly collagenous, fibers mentioned above, large elastic fibers also surround the alveolar mouths. The latter, however, occupy the outermost positions, lumenward from which nothing of a protoplasmic nature can be distinguished.

Alveolar pores

These structures were first seen quite incidentally in the course of this work in thick sections of lungs fixed by the intratracheal introduction of fluid and embedded in paraffin. Fearing that the heat shrinkage incident to such embedding might have been responsible for their presence, other lungs were embedded in celloidin and this technique subsequently used. Other experiments were run in the following ways: A young adult rabbit was killed, its chest opened and its trachea cannulated. After gentle inflation of both lungs with air, the root of one was firmly ligatured with silk. By means of the pressure bottles, already described, reversed to produce a gentle suction, the unligated lung was collapsed. Formol-Zenker was poured into the trachea and the second lung inflated with fixative to match the size of the first. The trachea was tied and the lungs removed and immersed in formol-Zenker. Next, to see whether the number of pores varies with the age of an animal, an old female rabbit was chosen. Her lungs were prepared in the same manner as were those of the young adult just described. Finally a longer series of lungs fixed by immersion after inflation, with air in some, with fluid in others, were examined for the presence of pores.

These structures appear as rounded or oval openings in the alveolar septa when the latter are viewed from their surfaces. They are most striking, and in fact are only to be seen when thick sections are deeply enough stained to render all tissue

substance definitely colored. They always occur in the meshes of the capillary latticework where the septa are thinnest, and frequently are bounded wholly or in part by the walls of vessels or by connective tissue fibers. Their margins are even and they are thus to be distinguished from ordinary tears. The latter, occurring in the course of the technique, are apt to be of any size and to appear anywhere, even cutting through blood vessels. Their margins are usually jagged and irregular and bear evidence of their origin.

In the air-inflated lungs of the young adult rabbits pores were seen but were not numerous. In young adult lungs fixed by the intratracheal introduction of fixative they were more numerous, but did not seem to differ in character. In the air-inflated lung of the old female they were a bit more numerous than in similarly prepared young adult lungs, while in her fluid-inflated lung they were very prominent. Here, as well as in sections from the block of mink lung, there were areas immediately beneath the pleura in which the alveolar walls when viewed from their surfaces consisted only of the capillary network. Between the vessels no tissue, save only an occasional naked fiber, was present: the interstices of the capillary net remaining as widely gaping pores. This, however, was an extreme condition and was not met in young adult lungs.

Ground substance

In routine sections of adult lungs stained with Mallory's technique it is possible to see something else not heretofore mentioned. This is the ground substance in which all of the previously described alveolar wall elements appear to be embedded. It appears as a palely staining, diaphanous, membrane-like substance, enveloping and cementing together the cells, fibers, and capillaries of which the septa are composed. Where pores occur it is entirely wanting. But here one gets the impression that in their formation a homogeneous membrane, under some elastic tension, has retracted from a point of rupture to the nearest supporting structures. The closest homology of this ground substance is with the so-called

homogeneous ground substance of the connective tissue, with which it may indeed be identical. Attempts were made to stain it differentially by means of the metachromatic reaction of toluidine blue, but with success only in rare instances. It was more definite in tissues fixed in alkaline reagents, and seemed thicker in lungs prepared by intratracheal instillation of ammoniacal silver carbonate.

SILVER METHODS

Silver nitrate instillation via the trachea

In order to see what the older writers had described as respiratory epithelium, lungs were prepared according to the directions of Elenz (1864) as modified by Bratianu and Guerriero ('31). A young adult rabbit was killed, its trachea cannulated and its chest opened. The pressure bottles, reversed to produce a gentle suction, were applied and the lungs deflated as much as possible. A solution of 0.2 per cent silver nitrate was gently poured into the trachea until the lungs were redistended to almost fill the chest cavity. The trachea was tied and the lungs removed and immersed in an excess of 0.2 per cent silver nitrate. They remained in this for 6 hours and were then transferred to 10 per cent formalin for 24 hours. Slices were cut, dehydrated, embedded, and sectioned to 75 μ to 90 μ . The method was also varied in this regard. In place of the silver nitrate solution there was used in another animal a solution of ammoniacal silver carbonate, prepared in the manner prescribed in the Foot reticulum technique, but with the excess ammonia combined by the addition of silver carbonate until a few grains of the precipitate barely failed to redissolve. But again as these two methods gave very similar results they will be described as one. Thick sections of this material were exposed to light to reduce the silver and studied either uncounterstained or lightly so with acridine red. They showed, in areas where bronchi or bronchioles were cut, a clear regular mosaic of small circular or irregularly polygonal areas surrounded by solid or dotted black lines, indicating the outlines of the lining

epithelium. In the respiratory portions, however, such pictures were not seen. Here the black lines seen on the surfaces of the alveolar septa were very irregular. Occasionally they completely outlined small rounded areas, but usually they simply ran for variable distances ending blindly in the septal tissue, sometimes intersecting one another.

Immersion of lungs in silver solutions

The method proposed by Chrzonszczewsky (1863) was also tried. This consists of the immersion of inflated lungs in weak solutions of silver nitrate. Young adult rabbits were killed, their chests opened and their tracheas cannulated. The lungs were inflated with air until they nearly filled the chest cavity, after which the trachea was securely tied. The whole lungs were removed and immersed for from 18 to 24 hours in solutions of 0.25 per cent silver nitrate. From these they were transferred to absolute alcohol for hardening and dehydration, then embedded in celloidin and sectioned at 75 μ to 90 μ . This technique was also used with the substitution of an ammoniacal silver carbonate solution, prepared as in the preceding paragraph, in place of the silver nitrate. Sections from the lungs immersed in silver nitrate, weakly counterstained with acridine red, appeared somewhat as follows: The greater part of the section, excepting a rim 2 to 4 mm. wide immediately beneath the pleura, was stained only with the red nuclear dye. Thus cell nuclei were red and cytoplasm a pale pink, under which conditions little in the way of structure could be seen. In the sub-pleural areas the silver had penetrated and presented a granular black deposit, sometimes quite indiscriminate, at others taking the form of vague lines in the tissue. There was a black mosaic outlined on the pleural surface, indicating the mesothelium. Nowhere could there be found areas such as Chrzonszczewsky figured in his plates, and it is not unlikely that what he saw was pleural mesothelium. Sections from lungs immersed in ammoniacal silver and counterstained with acridine red varied from the above in that the penetration of the silver was more complete. The

lines on the surfaces of alveolar septa were even more vague here than in the silver nitrate preparations.

Perfusions of lungs with silver citrate solutions

There was next attempted the method introduced by Bensley ('29) in his study of the vessels of the renal glomerulus. It is not the present intention to go into the details of that method but it may be briefly summarized as follows: An animal is first perfused with a hypotonic solution of sodium citrate sufficient to wash out the blood vessels. This is followed by a solution of silver citrate, made up according to formula. The tissue is fixed in formalin, embedded in paraffin, and cut in the usual manner. The sections are then developed with any good photographic developer and counterstained if desired.

Lungs, inflated with air, were prepared in this manner. Sections weakly counterstained with acridine red showed scattered blackened areas, which upon closer scrutiny were revealed as cross or longitudinal sections of small arteries and veins in which the lining endothelium was well defined. In places small branches could be seen to arise from these vessels, outlined in black for a distance of a few micra, where the impregnation appeared to cease. A few areas were to be seen in which on the surfaces of alveolar walls variable portions of the capillary net appeared to be outlined. The impregnation, however, in the capillaries seemed to outline not individual cells but rather the profiles of the vessels themselves. These preparations proved to be of additional value, as indicated above. It was found that if they were counterstained with Mallory's technique, then the capillaries of the septa appeared enough denser than the intervening tissue to be clearly visible. In such preparations, as already noted in the paragraph on capillaries, the locations of cell nuclei seen in alveolar walls could be accurately determined, and the identities of the endothelial cells thus established.

Newborn lungs

In order to see what changes, if any, occur in the respiratory portions of the lungs at birth, the following procedure was undertaken: A pregnant female guinea pig was observed until judged to be at term. She was then anaesthetized and her uterus clamped and removed. One uterine horn, containing one fetus, was clamped, cut away and immersed in formol-Zenker. The other horn containing two fetuses was opened and the young removed. One of these gasped a few times, but could not be resuscitated; the other promptly began breathing, its umbilical cord was tied and the animal was placed in an incubator at 37° C. The first uterine horn was opened under fixative and the fetus removed, never having been exposed to air. Two cubic centimeters of formol-Zenker were introduced into its trachea with a hypodermic syringe, partially distending its lungs. They were then removed whole to fixative. The second newborn—which gasped but did not live—was treated similarly. The third, which lived, was kept alive for 2½ hours. At the end of this time it was found dead on the uncovered incubator heating coil, and its lungs were fixed in a manner similar to that of the others.

Lungs of these series were embedded in paraffin and sectioned, some to 30 μ , others to 4 μ , and stained with Mallory's connective tissue stain. No differences could be detected between the lungs of the animal which had not breathed at all and those of the one which had gasped a few times. Thin sections from the lungs of these two animals were very interesting. They revealed the usual open lacework of structure with thin alveolar septa making up most of the tissue and dividing the larger spaces into smaller cubicles. Traversing the sections irregularly appeared the branches of the bronchi and bronchioles with their characteristic thick walls and tall ciliated epithelia. These, as in most descriptions, were accompanied by their corresponding blood vessels. The latter were of interest because they appeared only scantily filled with blood, as did also the finer vessels. Just as in adult lungs, where respiratory bronchioles could be seen and

the alveolar ducts derived from them followed, the usual sharp change in the character of the lining occurred, even though there had been no respiration. As the alveolar ducts emerged from the respiratory bronchioles, the tall, frequently ciliated epithelium of the latter immediately disappeared. Following the course of the ducts, the alveoli opening from them appeared, as usual, to lack completely any trace of an epithelial lining. In the alveolar walls there were to be seen a few scantily filled capillaries, apparently lying entirely naked to the alveolar lumens, separated from them only by their own endothelial walls. Indeed in some septa there were points at which the entire thickness consisted of a single capillary, cut across, lying seemingly naked to the alveolar lumens on either side of it. The cells of the alveolar septa were similar to those of adult lungs. Other than the capillary endothelium they consisted of cells largely limited to the regions of junction of septa. They were of rounded or roughly polygonal shapes, with regular rounded nuclei and granular appearing, somewhat vacuolated cytoplasm. They are the septal cells previously noted.

Thirty micra sections of the same lungs present much the same picture seen in 75μ to 90μ sections of adult lungs. There are two probable reasons for this: First, lungs at this stage are of very small size, and second, these were only partially distended with fixative. Probably owing to the first factor, and hence to the small size of the bronchial tree relative to the sizes of individual cells, the alveolar septa seem more cellular than in the adult. Alveolar pores were not seen, but little can be inferred from this owing to the incompleteness of inflation of the lungs.

Sections from the lungs of the animal that had lived for a short time failed to present any structural differences from those of its litter mates described above. There was, however, a striking difference in the completeness with which the blood vessels were filled. Whereas in the first two animals they had seemed almost empty, here they were well engorged and somewhat dilated.

DISCUSSION

The general picture of the embryologic development of the lungs has been well known for a long time. Quite recently Bender ('24) has made extensive studies of the subject and given excellent accounts of the fetal course. He has traced the growth and division of the primitive lung buds as they arise from the foregut, and has investigated in detail the formation and structure of the later branchings. His description, however, still leaves obscure the question of the changes which must occur in the epithelial linings of the ends of the bronchial branches if these are to give rise to the alveoli of the fully formed lung. Stewart ('23) has studied this subject and describes rarification occurring in the connective tissue surrounding the primitive bronchial endings, permitting them to expand. He also notes the probable formation of the non-nucleated plates of the adult respiratory epithelium by the elasmotosis of 'flanges' from large nucleated cells. Neither of these workers agree with the older workers, as Schmidt (1866), who thought that the cubical epithelium of the fetal respiratory surfaces was flattened to squamous by the first respirations of post-natal life. My own experiments with newborn guinea pigs further refutes this. However, neither Bender nor Stewart, nor Fauré-Fremiet and Dragoiu ('23), who have made extensive studies on the lung of the fetal sheep, account for the structure of the respiratory surface of the adult lung as does some still more recent work. Rose ('28) studied a series of human embryos from 5 months to term, by injecting fixing fluids into their tracheas and thereby distending their lungs. He describes from such preparations the appearance of faults between the cells lining the lower part of the bronchial tree, opening out directly into the spaces of the mesenchymatous tissue surrounding it. These spaces in the mesenchyme he would have as the future respiratory portions of the lung. His studies account exactly for the impression one gets from looking at a histologic section of adult lung, with the regular lining epithelium of the respiratory bronchioles appearing to end abruptly at the mouths of the alveolar ducts and alveoli.

The classical picture of a respiratory epithelium in the lung is based upon silver technique in which the outlines of cells are blackened when a tissue previously treated with silver nitrate is exposed to light. This technique also darkens the nuclei of cells thereby rendering them visible. From such technique was described the existence of the large non-nucleated plates and the small scattered nucleated cells. Such a technique is the only one which reveals such an epithelium. Furthermore, when the method is applied to the mammalian lung the result in the case of the respiratory surface is not the sharply outlined mosaic that is seen when silver nitrate is applied to other epithelial surfaces. The picture in the former shows often vaguely indicated, often not continuous, black lines which might well indicate many other things than cell outlines, as already suggested by Maximow-Bloom ('34), Bratianu and Guerriero ('31), and others. Thus the outlines of capillary walls and of alveolar pores, as well as of imperfectly impregnated connective tissue fibers, may well be here confused. Nor does the silver method distinguish between cell types. Even the cells within the blood vessels may be blackened and indicated by this means. Besides, anyone who has worked with silver techniques realizes how inconsistent and frequently undependable their results are. Miller ('32) who departs from the classical views and believes in a respiratory epithelium consisting of homogeneous squamous cells without the presence of non-nucleated plates invokes pathologic processes to demonstrate his views. He describes and figures lungs from cases of resolving pneumonia in which the alveolar epithelium is lifted off of the alveolar walls by a fluid exudate. But one might well go a step further along the line of reasoning he himself has used and suggest that his 'epithelium' as well as the fluid back of it is but exudate. For it has been amply demonstrated by Fried ('27, '28, '31), Gardner and Smith ('27), Bratianu and Guerriero ('31), and others, as well as above, that multitudes of histiocytes may rapidly appear in the alveolar septa. Also such arrangements of histiocytes are not uncommon along surfaces in tissue cultures.

The subject of the existence of alveolar pores has not received a great deal of attention. Kohn (1893) described some open communications occurring between adjacent alveoli through the interposed walls. In pneumonic lungs these were marked by the presence of fibrin threads traversing them. But he described them as also occurring in normal lungs. He quotes Adriani (1847) as having seen them earlier. Hansemann (1895) described them again and was able to reproduce them by the injection of colored masses into the tracheas of experimental animals. In these he demonstrated the presence of numerous threads of the injection masses passing through alveolar walls, and was convinced of the normal character of the pores thus indicated. Subsequently Bezzola (1894), Herbig (1894) and Ribbert (1894) also described and figured pores as occurring in pneumonia, but denied their existence in normal lungs. Aigner (1899) reproduced them by injecting carmin gelative masses into the tracheas of animals, as had Hansemann earlier. But unlike the latter, he maintained that he had produced artifacts by forcing his injection mass through the delicate alveolar walls and so manufacturing his pores. Von Ebner (1899) in Kölliker's *Handbuch* declared that pores were but artifacts, and Hansemann ('00) once more reaffirmed their normal existence. Miller (1893) denied the presence of pores in any of his corrosion or reconstruction preparations of the lungs of the dog, cat, rat, rabbit, sheep and human, and much later ('23, '25) further described their occurrence in pneumonia, but denied their existence in normal lungs. He ('23) described in detail the mechanism of their formation as depending upon the desquamation of the respiratory epithelium from diametrically opposed areas of alveolar septums, thereby exposing the capillary interstices of the walls as open faults. This view has been accepted by most subsequent authors, although Ogawa ('20) in his studies on the lungs of laboratory animals reported the presence of alveolar pores in his preparations; and Seemann ('31) regards them as normally occurring structures.

I do not wish to claim that alveolar pores are of normal occurrence in the strictest sense of the term, nor do I wish to admit that they are mere artifacts resulting from faulty preparations of lungs. A middle ground is defensible. Thus they are seen to be of only scant occurrence in the lungs of young adult animals, but more plentiful in older ones. They are also few in air-inflated lungs, but more numerous in lungs fixed by the intratracheal introduction of fluid. They therefore appear to result from the continually occurring traumata incident to everyday life, as well as from injury by necessary but perhaps over-violent methods of fixation. To understand Miller's failure (1893) to demonstrate pores in corrosion preparations of lungs, one must realize that Wood's metal, celloidin, and the other materials used to fill the air passages do not give accurate pictures of the details of finer structure. For the advancing tide of injection mass is influenced by surface tension and capillarity phenomena to such extent that the more delicate passages go entirely unfilled.

That pores are not to be seen in collapsed lungs is also readily explained. In this case, owing to the general retraction of the lung tissue, alveolar septa are so folded as to make it almost impossible to get one into position for a surface view.

The presence of such numerous pores in the lung of the mink is an interesting confirmation of the above views. For such might well be expected to result from the habits of this animal of holding its inspired air for long periods of time in the course of its submergences.

There can be no doubt, from the descriptions of earlier workers, that pores occur regularly in pneumonia. Even Miller, one of the staunchest opponents of their normality, freely admits their presence here.

Finally, the physiologic occurrence of pores in adult lungs and the readiness with which their numbers may be increased argue strongly against the existence of a continuous respiratory epithelium.

CONCLUSIONS

1. The black lines seen on alveolar septa in silver nitrate impregnations of lungs are susceptible of other explanation than the view which characterizes them as outlines of lining epithelial cells.

2. The occasional nuclei seen in alveolar walls in addition to those of the capillary endothelium belong to histiocytes and possibly other connective tissue cells.

3. The phagocytic cells which occur in such profusion in the alveoli in case of need are histiocytes, and of largely extraneous origin.

4. The alveolar walls, in addition to the capillary vessels and cells, consist of a membrane composed of reticular and elastic fibers and a homogeneous transparent ground substance. It is upon the latter that the continuity of the membrane depends.

5. Alveolar pores are seen sparsely in young adult lungs fixed while inflated with air. They are more frequent in older animals and those whose lungs were fixed by the intratracheal introduction of fluid. They thus appear to owe their existence to the traumata of everyday life as well as to those of fixation.

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PLATE 1

EXPLANATION OF FIGURE

1 2 mm. $\times$ 10 oc. Table level. Newborn guinea pig lung. 4μ section. Formol-Zenker fixation. Mallory's connective tissue stain. Alveolar duct opening out of respiratory bronchiole. Al, alveolar duct; Ep, epithelium lining respiratory bronchiole; Cap, apparently naked capillaries.

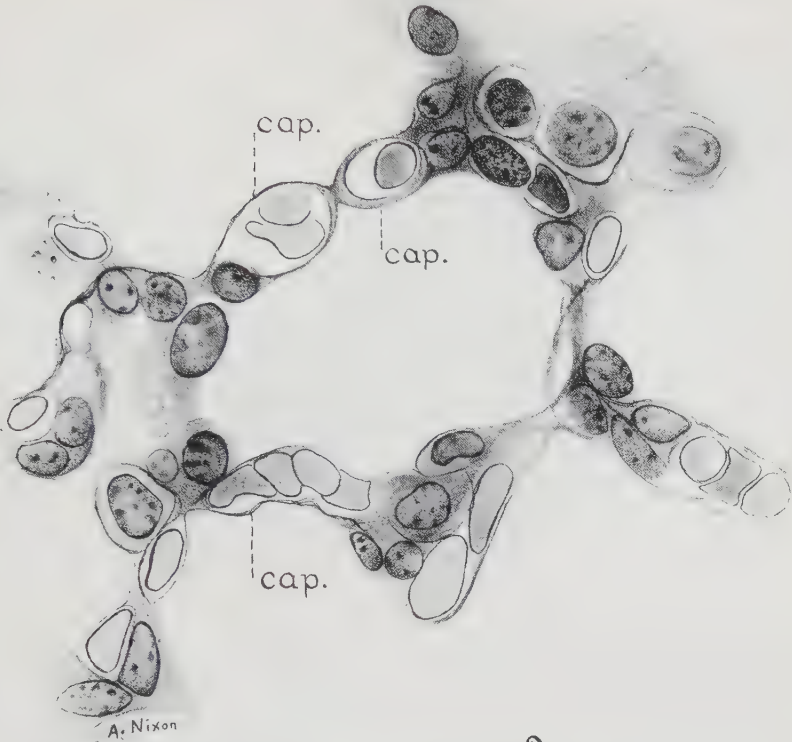


PLATE 2

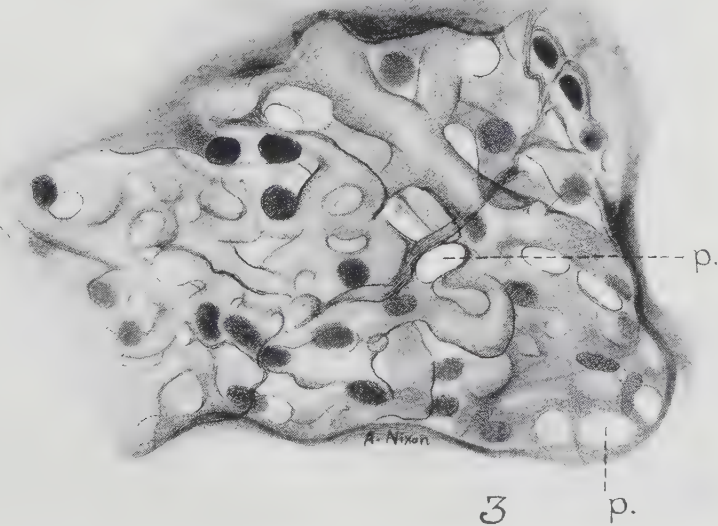
EXPLANATION OF FIGURES

2 2 mm. $\times$ 10 oc. Table level. Same lung as figure 1. 4μ section. Same stain. Single alveolus showing apparently naked capillaries in walls.

3 4 mm. $\times$ 10 oc. Table level. Adult rabbit lung. Animal perfused with silver citrate solution. Lung fixed in 10 per cent formol. 90μ section. Mallory's connective tissue stain. Surface view of alveolar walls showing capillary network and alveolar pores (p).



2



3

PLATE 3

EXPLANATION OF FIGURES

4 3 mm. $\times$ 10 oc. Table level. Same lung as figure 3. Same stain. 90 μ section. Surface view of alveolar walls showing capillary network and connective tissue fibers.

5 2 mm. $\times$ 10 oc. Table level. 100 μ section of lung of young mink. Lung fixed in Bensley's fluid intratracheally. Surface view of alveolus showing numerous pores (p).

